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Macrophage-Cytophilic Antibodies and the Functions of Macrophage-Bound Immunoglobulins

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INTRODUCTION

Although macrophage-cytophilic antibodies were first described over 10 years ago, very little progress has been made in the elucidation of their biological functions. The objects of this review are to summarize the information gained so far, to account for our relative lack of understanding of their biological functions, and to point out possible areas for future investigation.

Discovery and Definition

The first indications of the existence of macrophage-associated immunoglobulins were reported by Girard and Murray in 1954 (32). They detected antibodies in extracts of thoracic cavity macrophages and observed that these cells appeared able to concentrate passively introduced antibodies against *Salmonella*.

In 1960, Boyden and Sorkin (14) and Boyden, Sorkin, and Spärck (16) observed that certain rabbit antisera to human serum albumin (HSA), when mixed with normal rabbit spleen cells, fixed to these cells and, even after thorough washing, conferred upon them the capacity to specifically adsorb ^{131}I-HSA. In addition, similar properties were demonstrated in guinea pig antisera to HSA and in rabbit antisera to ovalbumin. Boyden and Sorkin (14) concluded that there was an antibody

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present in these antisera which had a strong avidity, not only for specific antigen but also for spleen cells. They therefore described this antibody as "cytophilic." Boyden (12) subsequently defined cytophilic antibody as "a globulin component of immune serum which becomes attached *in vitro* to certain cells in such a way that these cells are subsequently capable of specifically adsorbing antigen." This definition does not restrict the type of cell towards which an antibody may be cytophilic nor is any consequence presupposed to this adsorption and subsequent combination with antigen.

The most important cytophilic antibodies from a clinical point of view and hence the best investigated are the homocytotropic antibodies (4), cytophilic for mast cells and basophil polymorphonuclear leukocytes. These are usually detected by the release of pharmacologically active agents, such as histamine or serotonin, as a result of antigen-antibody interaction on or in very close proximity to the cell surface (92); although their biological function is ill understood, their clinical importance is well known.

The second major group of cytophilic antibodies are those which show a predilection for macrophage surface receptors and whose biological functions are only now beginning to be understood. These are the subject of this review.

There is evidence to indicate that antibodies cytophilic for neutrophil polymorphonuclear leukocytes also exist (29, 30) although little is known of their physicochemical properties or biological function.

Methods of Detection and Assay of Macrophage-Cytophilic Antibodies

Boyden and Sorkin (14, 16) successfully detected cytophilic antibodies by measuring the adsorption of isotope-labeled antigen to previously sensitized cells. Sorkin and co-workers (88–91) subsequently used this technique extensively in investigating the properties of these antibodies in rabbits. Apart from a requirement for isotope-handling facilities, this method also suffers from the inability of the investigator to identify the cells which adsorb antibody. Under some circumstances, it is also considered to be relatively insensitive (72).

A considerable advance was made in the study of macrophage-cytophilic antibodies when Boyden introduced a rosette-forming reaction on macrophage monolayers (12, 13).

Macrophage-monolayer technique. The cells under test, usually macrophages, are allowed to form monolayers on glass and, subsequently, the adherent cells are washed well and then exposed to antiserum. After sufficient time for cell sensitization has elapsed, the cells are again thoroughly washed and exposed to a suspension of antigenic particles. These may be erythrocytes, nucleated cells, or bacteria. After a final washing to remove unbound antigen, examination of the monolayers under the microscope reveals, in the case of a positive reaction, antigen rosettes around macrophages (Fig. 1 and 2).

Suspension-centrifugation technique. An alternative method for the assay of cytophilic antibodies to particulate antigens has been described by Jonas et al. (52). In this "suspension-centrifugation technique," a suspension of passively sensitized cells is mixed with appropriate antigen and the mixture is gently centrifuged. In a positive reaction, examination of the resuspended cell pellet reveals tight clusters of antigen particles around the test cells. This suspension-centrifugation technique may be used to detect antibodies cytophilic for cells which do not form monolayers or to test for rosette formation under conditions inimicable to monolayer formation. It is also said to be more sensitive than the macrophage-monolayer technique.

Modification of these techniques. The macrophage-monolayer technique and the suspension-centrifugation technique may be rendered still more versatile by the use of soluble antigen, conjugated to erythrocyte carriers. Jonas et al. (52) detected macrophage-cytophilic antibodies to bovine β-lactoglobulin and bovine plasma albumin in this way. Macrophage-cytophilic antibodies to bacterial antigens may be detected either by the use of erythrocytes conjugated with bacterial lipopolysaccharide or by the use of whole bacteria (6, 25, 79).

Sulica et al. (94) have used hapten coupled to phage T4 to detect anti-hapten cytophilic antibody. After fixation of antibody and phage-hapten conjugate to cells, excess hapten added to the system released the phage which could then be assayed.

Attempts to employ either antibody labeled with fluorescein isothiocyanate (52) or the mixed antiglobulin reaction (60) have been unsuccessful in the detection of cytophilic antibody.

Restrictions on the Use of the Term "Cytophilic" Antibody

The definition of cytophilic antibodies as proposed by Boyden (12) and quoted earlier explicitly states that the binding of antigen to cytophilic antibodies takes place subsequent to cellular fixation and implies, consequently, that these antibodies are capable of cellular fixation prior to their combination with antigen. Berken and

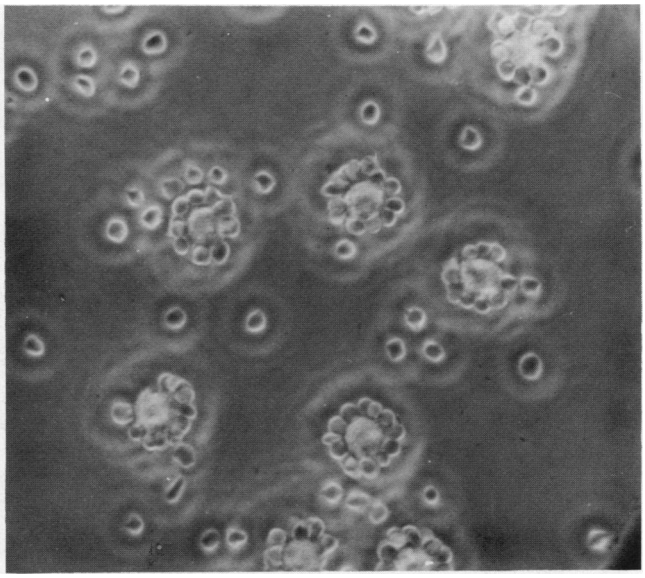

FIG. 1. *Sheep-erythrocyte rosettes around mouse peritoneal cells mediated by macrophage-cytophilic antibodies. Phase-contrast;* × *5,000.*

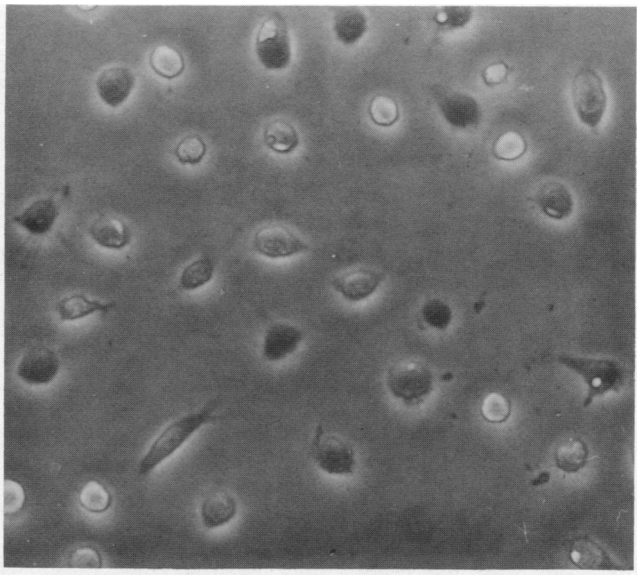

FIG. 2. *Mouse peritoneal cells exposed to sheep erythrocytes in the absence of macrophage-cytophilic antibody. Phase-contrast;* × *3,000.*

Benacerraf (5, 6) used the term "cytophilic" to include those antibodies which fixed to macrophages after combination with antigen and detected these antibodies by the "passive indirect technique." This technique involved reacting antigen with antiserum and, after washing the resulting antigen-antibody complexes, exposing macrophages in monolayers to them. When erythrocytes were employed as antigens, rosette formation morphologically indistinguishable from that due to cytophilic antibodies occurred. Berken and Benacerraf (7) considered cytophilia to be "that property of opsonizing antibody which provides the receptors that permit the binding of the antibody to the macrophage cell membrane in preparation for phagocytosis," thus defining both the cell involved and the consequence of such binding.

It has, however, been reported by Parish (79)

and later by Tizard (96) that the antibodies detected by this passive indirect technique, so-called opsonic adherence antibodies, are not cytophilic prior to combination with antigen and are therefore not identical to those antibodies detected by the macrophage-monolayer technique of Boyden.

Parish (79) was able to separate the opsonizing activity from the cytophilic activity of mouse anti-bovine plasma albumin and anti-bovine gamma globulin serum by agar-gel electrophoresis. Tizard (96) demonstrated that mouse anti-sheep erythrocyte cytophilic antibodies could be immunoglobulin (Ig)M, whereas the antibodies detected by the passive indirect technique are invariably IgG (7, 61, 96). As well as demonstrating that the two tests did not measure identical antibodies, Tizard (96) also showed differences between the two reactions in the types of cell involved; whereas cytophilic antibodies only bound to macrophages, opsonized erythrocytes in the passive indirect technique adhered to neutrophil polymorphonuclear leukocytes and to mast cells as well as macrophages. However, although these two reactions measure different antibody properties, they are also to some extent related. As will be discussed later, macrophage-cytophilic antibodies are readily eluted from mouse peritoneal macrophages at 37 C. Rosettes formed by macrophage-cytophilic antibodies on these peritoneal macrophages are stable at 37 C. Rosettes formed by the passive indirect method are also stable at 37 C. Erythrocytes sensitized by exposure to cytophilic antibody purified by elution from macrophages at 37 C can adhere to neutrophils and mast cells. This adherence is apparently identical to the adherence of erythrocytes sensitized with whole serum. There is thus evidence to suggest that once antigen has combined with cell-bound antibody, the resulting cell-antibody-antigen complex is indistinguishable from the structure formed by reaction of a cell with antigen-antibody complexes. This relationship does not detract from the observation that the passive indirect technique detects antibodies which are not cytophilic in the uncomplexed state and in vivo will not be detected in a cell-bound situation.

It is of interest to note that, whereas homocytotropic antibodies are cytophilic for mast cells in the absence of antigen, antigen-antibody complexes may adhere to these cells and, if the antigen is particulate, produce a rosette reaction rather than the release of pharmacologically active agents (95, 96).

Macrophage-Cytophilic Antibodies In Vivo

The amount of cytophilic antibody found in serum will depend upon both the avidity and availability of macrophage receptors. If the avidity or availability, or both, of these receptors is high, serum cytophilic antibodies may fall to undetectable levels.

Since macrophage-cytophilic antibodies do exist free in serum, it must be assumed that they either have a relatively low affinity for macrophage receptors or, alternatively, that all macrophage receptors are occupied. In either of these situations, molecules with a high avidity for macrophage receptors may be preferentially bound, and the possibility must be considered that the population of macrophage-cytophilic antibodies found free in serum represents those molecules with the lowest receptor avidity. These cytophilic antibodies detectable in serum may not, therefore, be typical of the population as a whole and may possess different biological properties to those antibodies of relatively high receptor avidity firmly bound to cells.

It is perhaps for this reason that, although serum macrophage-cytophilic antibodies are readily obtainable and much information is now available on their physicochemical properties, the nature of their biological function(s) is obscure. In contrast, where macrophages have been successfully examined for the presence of cell-bound antibody, it has been possible to show that they participate in relatively dramatic biological events. Therefore, the remainder of this review will be devoted to an examination of the macrophage-cytophilic antibodies detected in immune serum and those which are detectable only in the cell-fixed state and to a comparison of their properties.

MACROPHAGE-CYTOPHILIC ANTIBODIES DETECTABLE IN IMMUNE SERUM

Production and Physicochemical Characteristics

Rabbits. In the original experiments of Boyden and Sorkin (14, 15), antibodies cytophilic for spleen cells and directed against HSA were raised with the aid of Freund's complete adjuvant. Maximum titers, as detected by the binding of isotope-labeled antigen to cells, were found 1 week after a booster injection of antigen in saline. The primary injection of antigen in Freund's complete adjuvant was administered 2 weeks before the booster injection (6, 14–16). Antibodies to sheep erythrocytes may also be raised in this way (S. Kossard, Ph.D. Thesis, Univ. of Sydney, 1966).

Anti-sheep erythrocyte cytophilic antibodies

may also be produced in rabbits by a series of intravenous injections of antigen (65). Berken and Benacerraf (6) administered eight intravenous injections of sheep erythrocytes over a 2-week period and bled the animals 10 days later. Tizard and Soltys (102) detected cytophilic antibodies to *Trypanosoma brucei* 1 week after infection of rabbits with a mouse-adapted substrain of the organism. They employed the macrophage-monolayer technique utilizing intact trypanosomes as antigen.

Cytophilic antibodies in rabbits have been shown to be 7*S* IgG (89).

Guinea pigs. Boyden (13) found that the formation of cytophilic antibodies to HSA in guinea pigs required the mandatory use of Freund's complete adjuvant. This has been confirmed by others (1, 6, 52, 78, 84). High titers of macrophage-cytophilic antibodies were present in serum 3 weeks after a single dose of antigen in adjuvant and also 1 week after a booster injection in saline. Animals which received antigen incorporated in incomplete adjuvant or in saline did not produce these antibodies (52). When the effect of route of injection of antigen (sheep erythrocytes) was investigated (78), it was found that the subcutaneous route was much less effective than intradermal or intraperitoneal routes or injection into the footpads. However, although the subcutaneous route was ineffective in producing cytophilic antibodies, it was not deficient in inducing the formation of other types of antibodies, such as complement-fixing antibodies and hemagglutinins.

In contrast, cytophilic antibodies to HSA in guinea pigs (78) were produced to a similar extent after subcutaneous or intradermal administration in adjuvant. The difference presumably was due to the very different nature of the antigen. Maximum titers were present 14 days after intradermal inoculation but were undetectable 7 days and 3 months after immunization. Blazkovec (8, 10) and Holtzer (42) have produced cytophilic antibodies to soluble proteins in these animals by injection of immune complexes.

Amos et al. (1) have raised macrophage-cytophilic antibodies to purified protein derivative (PPD) tuberculin in guinea pigs by administering PPD in Freund's complete adjuvant to Bacillus Calmette-Guérin (BCG)-infected animals. Neither BCG nor PPD in complete adjuvant, when given alone, was particularly effective in this respect. Cytophilic antibodies have also been raised in guinea pigs to bovine β-lactoglobulin and bovine serum albumin (6, 52), to *S. typhimurium* (25), to encephalitogenic basic protein (31), and to dinitrophenol hapten (94).

Macrophage-cytophilic antibodies in guinea pigs have been shown by starch block electrophoresis (52), diethylaminoethyl cellulose chromatography (78), preparative ultracentrifugation (6), and gel filtration (8) to be confined to the 7*S* IgG_2 immunoglobulins (35). Lokaj and Mecke (65) claimed to detect 19*S* macrophage-cytophilic antibodies in guinea pig anti-sheep erythrocyte serum by chromatography on G-200 Sephadex (Pharmacia).

Mice. In contrast to the apparent necessity for the use of Freund's complete adjuvant in guinea pigs, cytophilic antibodies may be obtained without the use of adjuvant in mice. This is especially true of anti-sheep erythrocyte cytophilic antibodies (6, 62, 64, 96). Tizard (96) found that these antibodies could be produced by one or two intraperitoneal or intravenous injections of sheep erythrocytes in saline. These routes were found to be much more efficient than the subcutaneous route or injection of antigen intramuscularly in Freund's complete adjuvant (I. R. Tizard, Ph.D. Thesis, University of Cambridge, 1969). This lack of reactivity of mice to sheep erythrocytes incorporated in Freund's complete adjuvant was reported previously by Dietrich (26). Nelson and Mildenhall (77) obtained anti-sheep erythrocyte macrophage-cytophilic antibody in mice by inoculating sheep erythrocytes in saline intradermally 2 weeks after a first injection of erythrocytes in complete adjuvant. The titers of anti-sheep erythrocyte cytophilic antibodies also depend upon the strain of mice tested. Nelson (72, 75) has reported that C57Bl/6J mice, which normally have relatively high titers of naturally occurring anti-sheep hemagglutinins, may also be found to possess cytophilic antibodies to sheep erythrocytes in the absence of apparent previous sensitization.

Parish (79) has reported that mice can produce macrophage-cytophilic antibodies to *Escherichia coli* lipopolysaccharide. He found that cytophilic antibody production did not require the use of adjuvant but that, without it, antibody production was irregular. A single intravenous injection of 10^5 acetone-dried *E. coli* cells resulted in antibody production 5 or 6 days later. Antibodies could also be produced by a single subcutaneous injection of 10^5 acetone-dried organisms. Antibody activity increased by the effect of alum or Freund's complete adjuvant was nonspecific, since an increased response was obtained even when adjuvant was administered in a separate site from the antigen (W. E. Parish, *personal communication*). The formation of these antibodies in mice has also been reported by Mittal (K. R. Mittal, Ph.D. Thesis, University of Guelph, 1970).

Hoy and Nelson (45) reported the occurrence of macrophage-cytophilic antibodies in C57Bl/6J mice directed against alloantigens of the sarcoma I A/J tumor. These cytophilic alloantibodies were obtained by using either tumor cells or A/J skin grafts and were detectable by a macrophage-monolayer technique and rosette formation involving tumor cells around macrophages. After tumor implantation or a single skin graft, cytophilic alloantibodies reached maximum titers 14 days later.

Tizard (96) found that, after a single injection of sheep erythrocytes, mouse macrophage-cytophilic antibodies were predominantly IgM. After a second injection of antigen, the cytophilic antibodies were IgG (17, 96). Elution of cell-bound immunoglobulin (99) from macrophages treated with hyperimmune anti-sheep erythrocyte serum has revealed the eluate to have a fast gamma mobility, suggesting that these cell-bound immunoglobulins could be IgG_1.

Parish (79) reported that macrophage-cytophilic antibodies to *E. coli* were very unstable and probably IgM. Lokaj (62) observed that the mouse anti-sheep erythrocyte cytophilic antibodies observed by him were destroyed by 2-mercaptoethanol and that they could be either 7 or 19*S*. Nelson (73, 76) has shown that opsonic adherence activity for sheep erythrocytes occurs in the fast α-postalbumin region on Pevikon block electrophoresis, and some of his results indicate that cytophilic antibodies may occur in this region also. Parish (79) using agar-gel electrophoresis demonstrated that mouse macrophage-cytophilic antibody to BSA or BCG migrated in the slow gamma region, i.e. was IgG_2. Askenase (Fed. Proc., **30**:Abstr. 2787, 1971) could inhibit the reaction of mouse anti-oxazolone cytophilic antibodies to antigen by treatment with antiserum to mouse gamma globulin, mouse light chains, or mouse $gamma_{2a}$ or $gamma_{2b}$ determinants. Macrophage cytophilic antibodies in mice are not present in IgG_3 (39).

Rats. Sodoman and Haferkamp (85) reported that rats can produce macrophage-cytophilic antibodies to tuberculoprotein after infection with BCG. Injection of PPD alone did not induce cytophilic antibody formation (see also reference 1). They also reported the occurrence of cytophilic antibodies to group A streptococcal carbohydrate (86). Cytophilic antibodies have been reported to occur in rats with experimental allergic encephalomyelitis (31). These are apparently directed against the encephalitogenic basic protein.

Poultry. There have been several reports of the occurrence of cytophilic antibodies in poultry sera (66, 87); however, since they deal with opsonic adherence and involve the passive indirect technique, they will not be discussed further here. Ivanyi (51) has shown that material present in hen anti-human serum albumin was cytophilic for calf spleen cells.

Primates. Kay et al. (53) raised macrophage-cytophilic antibodies to rabbit erythrocytes in baboons, and Lascelles et al. (59) demonstrated that these were cytophilic not only for baboon cells but also for human colostral macrophages. Inchley et al. (49) have surveyed human myeloma proteins for cytophilic activity and demonstrated that it was present only in myelomas of the IgG_1 type, as detected by their capacity to bind to guinea pig macrophages and inhibit adsorption of guinea pig macrophage-cytophilic antibodies.

Binding of Serum Cytophilic Antibodies to Macrophages

Binding site on the antibody molecule. Berken and Benacerraf (6) demonstrated that pepsin treatment of guinea pig macrophage-cytophilic antibodies destroyed the capacity to fix to cells. A similar finding has been reported for mouse cytophilic antibodies (96).

Since pepsin acts on immunoglobulin molecules by digesting the Fc portion of the antibody heavy chain, it is probable that this is the region involved in cell fixation. There is unfortunately little evidence to indicate the exact nature of this binding site. Inchley et al. (49) demonstrated that, whereas human $gamma_1$ myeloma proteins were cytophilic, $gamma_2$ myeloma proteins were not, and yet peptide mapping has yielded only four peptides which differ between these two subclasses (40).

Opsonic adherence antibodies become able to fix to cells only after combination with antigen and such a combination has been shown to induce conformational changes in the IgG molecule (19, 50, 70). It is perhaps the preexistence of these changes in an uncomplexed molecule which renders it cytophilic. Similar changes probably occur in the Fc portion of homocytotropic antibody molecules, thus permitting cellular fixation to mast cells to occur (93).

Macrophage membrane receptor for cytophilic antibodies. Howard and Benacerraf (44) and Davey and Asherson (23) investigated the nature of the macrophage receptor for cytophilic antibodies in guinea pigs. They found that these receptors were insusceptible to attack by proteolytic enzymes (trypsin, chymotrypsin, papain, ficin, and Pronase) but were susceptible to phospholipase A, lecithinase C, *Naja naja* venom, and agents reacting with phospholipids and -SH groups. Kossard and Nelson (57) also observed that trypsin and pepsin did not destroy receptors for 7*S* IgG cytophilic antibodies on mouse and

guinea pig macrophages but could enhance antibody uptake. Both Nelson and Boyden (75) and Tizard (96) have observed that the receptor for mouse 19S cytophilic antibody is trypsin-sensitive, and Sorkin (90) observed that trypsin-treated rabbit macrophages cannot take up spleen cell cytophilic antibodies.

Cells to which cytophilic antibodies are adsorbed. Much of the early work performed by Boyden and Sorkin is difficult to evaluate with regard to the cell type involved since they employed an isotope technique and could not, therefore, identify reacting cells with accuracy. Sorkin (90), however, reported that the rabbit cytophilic antibodies which he detected could bind to granulocytes and lymphocytes and Keller and Sorkin (54) claimed that the antibody which they examined was cytophilic for rat tissue mast cells and liver cells.

Boyden (13) was the first to confirm that antibodies specifically cytophilic for macrophages existed. It has since been found (44, 52, 96) that mouse and guinea pig sera containing macrophage-cytophilic antibodies did not sensitize neutrophils, eosinophils, lymphocytes, fibroblasts, peritoneal serosal cells, alveolar epithelium, mucosal epithelium, or mast cells. In the mouse, it was also observed (96) that alveolar, splenic and peritoneal macrophages, Kupffer cells, microglia, and blood monocytes took up cytophilic antibodies to an apparently identical extent. Berken and Benacerraf (6) have reported that mouse, guinea pig, and rabbit alveolar macrophages adsorbed more cytophilic antibodies than their peritoneal cells. It has also been observed (22) that guinea pig small lymphocytes, transformed in mixed cell culture, may also take up macrophage cytophilic antibodies. Kossard (Ph.D. Thesis, University of Sydney, 1966) found that rabbit anti-sheep erythrocyte antisera contained antibodies cytophilic for rabbit and guinea pig macrophages and small lymphocytes.

Several workers have investigated the interspecies specificity of these reactions, producing interesting results but little of general biological significance (6, 56, 65).

Factors Which Affect the Binding of Macrophage-Cytophilic Antibodies to Cell Receptors

Effect of different temperatures. The effect of the ambient temperature upon cytophilic antibody uptake appears to vary between species. However, most adsorptive processes, probably because of the considerable entropy changes involved, are usually inhibited by a rise in temperature.

The adsorption of rabbit anti-HSA was thus found to be greater at 0 C than at 37 C, at which temperature some bound antibody was eluted (10), although it has also been reported (N. R. Rose and R. C. Brown, Fed. Proc. **21**:44, 1962) that temperature has little effect on rabbit cytophilic antibody uptake. Whereas Jonas et al. (52) observed that changes in temperature did not significantly affect guinea pig macrophage-cytophilic antibody adsorption, Berken and Benacerraf (6, 7) observed that it proceeded better at 37 C than at room temperature or 4 C, and Kossard and Nelson (56) and Boyden (12) reported that the reverse was the case. Tizard (97, 99) observed that both mouse IgG and IgM macrophage-cytophilic antibodies were bound to cells much more strongly at 4 C than at 37 C.

Competition for receptors from "nonspecific" cytophilic immunoglobulins. The rosette-forming reaction, as measured by the macrophage-monolayer technique, is profoundly affected by the presence, in the serum under test, of cytophilic antibodies directed against antigens other than those specifically looked for. These nonspecific cytophilic antibodies are able to compete against the specific antibodies under test for available cell receptors. For this reason, the addition of normal serum to diluent is normally inhibitory for cytophilic antibody rosette formation and, similarly, rosette formation as measured by this test reflects the result of this competition for available receptors (6, 49, 52, 56, 63).

Heterogeneity of cell surface receptors. The adsorption of cytophilic antibodies onto macrophages is but one example of the adsorption of solute onto a surface. These solute-surface reactions are, in theory, governed by the relationships encompassed in the Langmuir adsorption isotherm. This is a mathematical expression indicating that the amount of solute adsorbed by a surface is proportional to the amount available for adsorption until the surface receptors are fully occupied. When the amount of IgG cytophilic antibody adsorbed by mouse macrophages was measured, either by use of a radioactive label or by counting adhering erythrocytes, and this uptake was plotted against the amount of antiserum presented to the cells, it was found that the adsorption deviated from the theoretical curve (100). This deviation was not observed in the adsorption isotherms of mouse IgM cytophilic antibody nor of opsonized erythrocytes.

Deviations from theoretical isotherms may be due to interaction between receptors. Since the presence of very low levels of nonspecific mouse IgG was found to enhance cytophilic antibody adsorption both in vitro and in vivo, it is probable that this receptor interaction is cooperative in nature. If this is in fact the case, a net movement of antibodies onto cell receptors may then occur

under conditions of raised immunoglobulin levels such as in inflammatory foci. It has been observed that mouse peritoneal cells take up considerably more cytophilic antibody 48 hours after intraperitoneal injection of oil than do cells from the unstimulated peritoneal cavity (100).

Effect of bacterial lipopolysaccharide on cytophilic antibody uptake. Bacterial lipopolysaccharide (endotoxin) mediates a wide variety of biological phenomena, many of which involve the reticuloendothelial system and which are difficult to interrelate. It is known, for instance, that these bacterial lipopolysaccharides have a profound effect upon macrophage-cytophilic antibody uptake from serum by mouse macrophages. A single injection of *E. coli* lipopolysaccharide intravenously into mice affects the peritoneal macrophages of treated animals so that their capacity to adsorb cytophilic antibodies is altered.

Five minutes after a single injection, antibody adsorption is enhanced; 30 min after the injection, it is profoundly depressed; and, from about 6 to 48 hr after the injection, the macrophages can take up an increased amount of antibody (98). These changes are unrelated to the metabolic activity of these cells but are apparently mediated by a factor or factors in the serum of treated animals. Changes in the levels of nonspecific cytophilic antibodies are not responsible for the effect since the response may take place in gnotobiotic mice (101).

Biological Significance of Serum Cytophilic Antibody

In spite of their obvious similarities to homocytotropic antibodies, macrophage-cytophilic antibodies as detected in serum are not anaphylactic (11). There has been a report that cell-associated immunoglobulin can protect macrophages against cytotoxicity due to endotoxin (R. W. I. Kessel, W. Braun, and O. J. Plesica, Fed. Proc. **23**:564, 1964). The relationship of this immunoglobulin to macrophage-cytophilic antibody is obscure.

There is, however, evidence that macrophage-cytophilic antibodies may be opsonic. Tizard (97) passively sensitized mouse macrophages with immunoglobulin fractions of anti-sheep erythrocyte serum. After sensitization at 4 C, the cells were washed, antigen was added, and the mixture was incubated at 37 C. It was observed that both IgM and IgG cytophilic antibodies enhanced phagocytosis of sheep erythrocytes but that the IgM-containing fraction was less effective in this respect than the IgG-containing fraction. Since cytophilic antibodies in mice are eluted from macrophages at 37 C, it was difficult to ascertain the degree of sensitization of these macrophages and whether the fact that the antibodies involved were cytophilic affected the degree of phagocytosis. It is, however, well established that some cytophilic antibodies may fix antigen to macrophages without inevitably stimulating phagocytosis. Levenson and Braude (60) observed that anti-*S. typhi* Vi polysaccharide obtained early in immunization could induce rosette formation of Vi-coated erythrocytes around macrophages and that prolonged incubation of these rosettes at 37 C did not lead to phagocytosis. A similar situation may exist in the primary follicles of mammalian lymph nodes. Within these primary follicles are the dendritic reticulum cells, a group of cells which are usually considered to be reticuloendothelial but are distinguishable from the macrophages found in the medulla both morphologically and functionally. Mitchell and Abbott (69) found that, although the macrophages of the lymph node medulla phagocytosed antigen in the usual way, the reticular cells in the germinal centers did not; instead they localized it on their extensive dendritic processes which are in close proximity to lymphocytes. This antigen localization was found by Humphrey and Frank (47) to occur only when antibody started to appear. It did not occur in tolerant animals and was very marked in primed animals. These findings suggested that an antibody, cytophilic for dendritic reticular cells, was responsible for antigen localization in lymph node primary follicles. Unfortunately, the relationship of these cells to "classical" macrophages is not known.

An alternative role for serum cytophilic antibodies has been proposed by Ivanyi (51). He used a hyperimmune chicken anti-HSA serum which he deprived of its cytophilic antibody content by adsorbing six times with calf spleen cells. The adsorbed cytophilic material was isolated by warming the spleen cells to 56 C for 1 hr, thus eluting any cell-bound material from them. The three preparations, untreated antiserum, adsorbed antiserum, and eluate, were tested for their capacity to inhibit a primary response to HSA in chickens. Ivanyi found that, although the untreated antiserum completely suppressed the primary anti-HSA response, the absorbed antiserum and eluate enhanced the primary response. Ivanyi interpreted this to indicate that the cytophilic antibodies in this anti-HSA serum were responsible for inhibition of the primary response. The lack of suppressive activity in the cell eluate was considered to be due to the high dilution of its contained cytophilic antibody. There is at present no adequate explanation for this phenomenon and the possible biological role for macrophage-cytophilic antibody detectable in serum is still shrouded in a mass of conflicting reports despite the minutiae of detail available

on its physicochemical characteristics. The reverse situation is true for those immunoglobulins detected upon the surface of macrophages derived from immune animals.

CELL-BOUND CYTOPHILIC ANTIBODIES

In 1949 Burnet and Fenner (18) suggested that delayed hypersensitivity, a type of specific cell-mediated immunity, might be due to an antibody which had a strong affinity for cells. If all of this antibody were cell-bound, it would never reach a high enough concentration in serum to permit serum transfer of immunity.

While evidence accumulates that some form of cell-bound antigen-specific material plays a role in delayed hypersensitivity, there is little to suggest that it is identical to serum macrophage-cytophilic antibody. Nelson and Mildenhall (77) claimed that a correlation existed between levels of serum cytophilic antibody to sheep erythrocytes in mice and the delayed footpad reactions to these antigens. However, such relationship as exists between these two reactions may be due to coincidence between peak levels of serum cytophilic antibodies and delayed skin reactions, especially if Freund's complete adjuvant is employed to raise the cytophilic antibodies.

The bulk of available evidence indicates that no relationship exists between serum macrophage-cytophilic antibody levels and the intensity of delayed skin reactions (9, 36, 41, 43, 78) Holtzer (42) produced delayed hypersensitivity to sheep erythrocytes in guinea pigs in the absence of detectable serum cytophilic antibody and demonstrated that the skin reactions were not enhanced by local injection of macrophages or macrophages sensitized with cytophilic antibody in vitro. Nor was the reaction enhanced by infiltration of the site of antigen injection by serum containing cytophilic antibodies.

There is, in contrast, an increasing body of evidence that indicates that cytophilic factors, separate from serum cytophilic antibody, do exist and that they may passively sensitize macrophages which consequently may participate in the reactions of cellular immunity, delayed skin reactions, allograft rejection, and perhaps even acquired cellular immunity to intracellular microorganisms.

Cytophilic Factors in Delayed Hypersensitivity

The earliest report of the existence of such cytophilic factors was by Hulliger et al. (46), who passively sensitized normal guinea pig peritoneal exudate cells with serum from guinea pigs immunized with sheep erythrocytes in Freund's complete adjuvant. When these sensitized cells were injected, together with antigen, intradermally into normal recipients, a skin lesion developed at the injection site which reached maximum severity 24 hr after injection. Injection of antigen together with serum from hypersensitive animals gave rise to an Arthus-type reaction with maximum severity between 4 and 12 hr. The transferred exudate cells were shown to be producing small amounts of anti-sheep erythrocyte antibody, but this appeared to be unrelated to either the severity or duration of the lesion. Thus, these experiments indicated that a lesion resembling that of delayed hypersensitivity might be mediated by a factor in serum having an affinity for cells in peritoneal exudate.

A report by Dupuy et al. (28) also indicated that a cytophilic factor, by passively sensitizing transferred cells, may play a role in delayed skin reactions. These workers utilized the observation that factors are apparently released into serum as a result of X irradiation and that these factors may be able to mediate cellular immune functions such as graft rejection (20, 21.) Dupuy and his co-workers were able to transfer delayed skin reactivity to normal guinea pigs by means of small quantities (2 to 3 ml) of fresh plasma from X-irradiated hypersensitive donors. (Tuberculin PPD was employed as antigen.) Plasma from nonirradiated PPD-sensitive donors or from X-irradiated nonsensitized donors did not transfer any skin reaction. When 3×10^8 viable nucleated spleen cells from normal guinea pigs were incubated with 5 ml of plasma from irradiated sensitized donors and after washing were injected into normal guinea pigs, the recipients were found to exhibit delayed skin reactions to tuberculin PPD when tested 6 days later. The spleen cell population was not defined, but in this case it appears that factors cytophilic for spleen cells or a subpopulation of spleen cells could render these animals hypersensitive.

The third group of experiments indicating the existence of, and a role for, cell-bound cytophilic immunoglobulins was performed by Zembala and Asherson (104). These workers induced contact sensitivity to oxazolone in mice. This is a mixed reaction with evidence for involvement of both delayed- and immediate-type immune reactions. Zembala and Asherson were able to transfer this contact sensitivity by means of serum from sensitive to normal mice. Contact sensitivity could also be transferred by normal purified peritoneal macrophages passively sensitized with immune serum. Passively sensitized lymphocytes could not transfer reactivity. Macrophages derived from actively sensitized mice could transfer reactivity, and this transfer could be abolished by trypsin treatment or by incubation of cells in anti-mouse gamma globulin

serum. These results strongly indicated that contact hypersensitivity in this situation was mediated, at least in part, by macrophage-bound immunoglobulin. Since skin reactivity to oxazolone is a mixed reaction, it would be important to determine which of the components, immediate or delayed, is transferred by these sensitized macrophages. Similar findings have been reported by Askenase (Fed. Proc. **30:** Abstr. 2787).

These three groups of investigations (28, 46, 104), although atypical in that in two of them serum or plasma could also transfer the reactions, constitute the most direct evidence for involvement of cell-bound immunoglobulins in delayed skin reactions. However, certain other observations may be linked to this reaction. It is now considered that delayed hypersensitivity in vivo may be reflected in vitro by the inhibition of macrophage migration in the presence of antigen and lymphocytes from sensitive animals. Amos et al. (1) raised serum cytophilic antibodies to tuberculin PPD in guinea pigs and observed inhibition of macrophage migration in the presence of antigen and these cytophilic antibodies. Dumonde (27), using sheep erythrocytes as antigen, has observed a similar phenomenon.

A second immunologically specific cytophilic factor has been reported by Amos and Lachmann (2, 58). This is not immunoglobulin, yet in the presence of specific antigen it will inhibit macrophage migration. The relationship of this material to cell-bound cytophilic immunoglobulin is not understood although presumably, under conditions where immunoglobulins are adsorbed by cells, this factor may also be adsorbed. There is one other similarity between macrophage migration inhibition factors (MIF) and macrophage-cytophilic antibodies, and this is their site of action. Cells treated with trypsin become insensitive to MIF but will regain their susceptibility upon incubation (3, 24). One possible explanation of this phenomenon is that MIF may act through trypsin-sensitive cell surface receptors which are capable of regeneration. These could be on the macrophages.

Nelson and Boyden (74) observed in 1961 that a subcutaneous injection of tuberculin PPD into BCG-infected guinea pigs resulted in the almost complete disappearance of macrophages from the peritoneal cavity of these animals; they considered this to be a manifestation of delayed hypersensitivity. Nelson (71) was able to transfer this form of reactivity to normal animals by means of normal peritoneal macrophages passively sensitized with serum from hypersensitive animals indicating that a cytophilic factor may play a role in this aspect of delayed hypersensitivity also.

Cytophilic Factors in Allograft Rejection and Autoallergic Reactions

Allograft rejection is to a large extent mediated by cells with immunological capabilities. One of the mechanisms of graft destruction is by a cytotoxic mechanism mediated through contact between "immune" host cells and graft cells. These cytotoxic reactions (34, 82) appear to be largely mediated by cells of the small lymphocyte type. However, Granger and Weiser (37) reported that sensitized C57Bl/Ks mouse peritoneal macrophages, upon contact with foreign cells (sarcoma I, derived from A/J strain mice), destroyed them. They demonstrated that the adherence between target cell and macrophage was mediated by a cytophilic factor which could be eluted from macrophages by heating them to 56 C. This factor agglutinated A/J mouse erythrocytes and was later shown to fix complement in a cytotoxic reaction against the target cells (38). Similar findings have been reported by Gordon (35). Weiser and his colleagues (103) have since demonstrated that activated macrophages could be "disarmed" by trypsin treatment and "rearmed" by exposure of these treated cells to either immune serum or eluates from activated macrophages. Weiser et al. (103) have also shown that, although macrophages activated against sarcoma I cells could be made to adhere to unrelated cells, i.e., Balb/c and C57BL/Ks fibroblasts, cell destruction did not occur.

A similar observation has been made by Hoy and Nelson (45) who raised macrophage-cytophilic antibodies in C57BL/6J mice against A/J strain grafts either by the use of sarcoma I cells or by A/J skin grafts, and, although they could induce adherence of macrophages to tumor cells, cytotoxicity was never observed.

Cell-bound cytophilic antibodies may also play a role in autoallergic reactions. Pokorna (81) using guinea pigs has prepared eluates from sensitized spleen, lymph node, and peritoneal exudate cells; he passively sensitized normal cells with these eluates and used these passively sensitized cells to transfer autoimmune aspermatogenesis to normal recipients. It was possible, however, that these eluates contained antibody synthesized by lymph node or spleen cells since eluates from these tissues were relatively more effective than eluates from peritoneal cells. Koprowski and Fernandes (55) showed that lymph node cells derived from rats immunized with dog brain tissue in Freund's complete adjuvant could adhere to cultures of puppy glia. They also demonstrated that lymph node cells from normal animals passively sensitized with

serum from immune animals also caused adherence, perhaps indicating the presence of some form of lymphocyte-cytophilic antibody.

Gibbs et al. (31) have demonstrated the presence of cytophilic antibodies to encephalitogenic basic proteins in the sera of guinea pigs and rats with acute experimental allergic encephalomyelitis (EAE). They also found antibodies in the serum of animals protected against EAE and were unable to transfer the reaction by the use of passively sensitized macrophages. They concluded that cytophilic antibodies only represented a concomitant reaction to sensitization with basic proteins and that the cytophilic antibodies played no significant role in the condition. It is, however, pertinent to note that they detected only serum macrophage-cytophilic antibody, which for the reasons outlined previously is unlikely to have a significant role in the cell-bound state.

Cytophilic Factors and Resistance to Infection

The third major form of cell-mediated immune response is the state of acquired "cellular immunity." This is manifested by an increase in the phagocytic and bactericidal abilities of macrophages and usually arises as the result of infection by an organism capable of intracellular multiplication such as *Mycobacterium tuberculosis* or *Listeria monocytogenes* (68). Infection with such an organism stimulates the appearance of a new population of small lymphocytes. These cells migrate rapidly to the site of infection, where, after interaction with antigen, they are able to influence the surrounding macrophages so that they are more capable of combating the invading organism (67). The induction of this lymphocyte population and its further interaction with antigen are immunologically specific. However, the modification of macrophages as a result of this interaction is nonspecific. Stimulated macrophages exhibit enhanced phagocytosis and destruction of any antigen presented to them. For this reason it is improbable that a macrophage-cytophilic antibody mediates these changes in macrophage behavior.

There is, however, evidence that some cell-bound immunoglobulins may act as opsonins (83). Immunity to *S. typhimurium* in mice may be transferred to normal animals by peritoneal macrophages from immune donors. Resistance may also be transferred by normal cells passively sensitized with serum from immune mice. Cells from immune donors were unable to transfer resistance after trypsin treatment. It was also found possible to elute material from pooled "immune" macrophages. This material was destroyed by 2-mercaptoethanol, was a macroglobulin, and was able to protect normal mice. In this instance, it is probable that the cytophilic material acted as classical opsonin rather than as a mediator of cellular immunity.

Biological Significance of Cell-Bound Macrophage-Cytophilic Antibodies

Cell-bound cytophilic antibodies render the cell which they sensitize capable of binding to a specific antigen. This binding of antigen to macrophage may be the only function of the antibody and any subsequent cellular activity may be unrelated to the presence of the specific antibody. Such a situation appears to occur in the cytotoxic reactions mediated by macrophages (45, 103). Cytophilic antibody can cause foreign cells to adhere to macrophages. The subsequent cytotoxic effect is apparently mediated directly from the cell and not through the cell-bound antibody.

The process could be as follows: Step 1. Antigen + cell-bound antibody → fixation of antigen. Followed by step 2. Fixed antigen + immune macrophage → cytotoxic effect.

Alternatively, the combination of antigen with cell-bound antibody may directly affect macrophage enzyme systems, i.e., antigen + cell-bound antibody → activation of macrophage enzyme systems → subsequent change in macrophage activity.

There are examples of such a situation on other cells leading to relatively dramatic cellular events. The combination of antigen with cell-bound homocytotropic antibody leads to release of pharmacologically active agents (92) and dramatic changes in cellular morphology (48). The combination of antigen with antibody-like receptors on antigen-sensitive small lymphocytes leads to blast transformation (80). No observations have been recorded of changes occurring in macrophages as a result of the binding of cytophilic antibody from serum. Perhaps these antibodies are insufficiently avid for cell receptors to activate membrane enzymes. There are, however, at least two examples of these changes occurring as a result of combination of antigen with cell-bound cytophilic immunoglobulin. One of these is the inhibition of macrophage migration by cytophilic antibody in the presence of antigen (1). The other is the enhancement of immunity, probably due to the phagocytosis observed by Rowley et al. (83). Other consequences will probably be revealed as the role of cell-bound antibody in delayed skin reactions is further investigated.

CONCLUDING STATEMENT

Our present knowledge of macrophage-cytophilic antibodies may be summarized by suggesting that current methods of detection make them fall into two groups. This division is related to their avidity for macrophages in vivo and is reflected in our understanding of their biological function.

Cytophilic antibodies detectable in serum probably represent that segment of the total cytophilic antibody population with a low avidity for cell receptors. Their physicochemical properties, the properties of the corresponding receptors on macrophages, and the factors affecting antibody fixation have been exhaustively studied; however, no clear understanding of their biological function has emerged.

In contrast, immunoglobulins detectable in the cell-bound state appear to be associated with the cell-mediated immune reactions; delayed hypersensitivity and cell-mediated cytotoxicity. The lack of association between these cell-mediated immune reactions and the presence of serum macrophage-cytophilic antibody may indicate that these two groups may represent not the extremes of a single variable population but perhaps a true functional heterogeneity among cytophilic immunoglobulins.

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